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Th1 – Th2 polarisation and autophagy in the control of intracellular mycobacteria by macrophages

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Abstract

Autophagy is a major intracellular pathway for the lysosomal degradation of long-lived cytoplasmic macromolecules and damaged or surplus organelles. More recently, autophagy has also been linked with innate and adaptive immune responses against intracellular pathogens, including *Mycobacterium tuberculosis*, which can survive within macrophages by blocking fusion of the phagosome with lysosomes. Induction of autophagy by the Th1 cytokine IFN- γ enables infected macrophages to overcome this phagosome maturation block and inhibit the intracellular survival of mycobacteria. Conversely, the Th2 cytokines IL-4 and IL-13 inhibit autophagy in murine and human macrophages. We discuss how differential modulation of autophagy by Th1 and Th2 cytokines may represent an important feature of the host response to mycobacteria.

Keywords

Autophagy; Tuberculosis; Th1; Th2; IL-4; IL-13; IFN- γ

1. Introduction

Tuberculosis is a disease of considerable social and economic impact globally. The primary route of infection is through the inhalation of aerosolized droplets of *Mycobacterium tuberculosis* or *M. bovis*. The bacteria ultimately reach the distal regions of the lung, where they are phagocytosed by macrophages and dendritic cells. In most cases the infection is contained after formation of a granuloma, the culmination of a T-helper 1 (Th1)-biased adaptive immune response. The resulting latent infection can be re-activated when the host immune response is compromised, such as during HIV infection or in old age. Understanding the processes involved in granuloma formation and containment of the bacteria is essential in the development of new treatments and vaccine strategies against tuberculosis. Here we examine how autophagy, a potentially vital facet of the innate immune response to mycobacteria, is differentially modulated by Th1 and Th2 cytokines.

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Conflict of interest

None.

2. Role of cytokines in the immune response to mycobacteria

Immunity to tuberculosis is associated with a predominantly Th1 cell-mediated response, characterised by the localised production and release of IFN- γ , IL-12 and TNF- α (Salgame, 2005). Genetic defects in the receptors for IL-12 and IFN- γ increase susceptibility to progressive mycobacterial disease (de Jong et al., 1998; Jouanguy et al., 1996, 1999), while treatment with TNF- α -specific monoclonal antibodies, used in the treatment of inflammatory disorders such as rheumatoid arthritis and Crohn's disease, can lead to reactivation of latent tuberculosis (Keane, 2005; Keane et al., 2001). Mice genetically deficient in IFN- γ (gko mice), although able to develop granulomas, fail to produce nitric oxide (NO) and are unable to restrict intracellular growth of the bacilli (Flynn et al., 1993). IL-12 also increases protection against mycobacterial infection in susceptible BALB/c mice, but not in gko mice, demonstrating that the protective effects of IL-12 are dependent on IFN- γ (Flynn et al., 1995). Similarly in cattle, induction of protective immunity against *M. bovis* infection is associated with the stimulation of high levels of IFN- γ (Hope and Vordermeier, 2005). *In vitro*, *M. bovis* induces strong IL-12 secretion in bovine dendritic cells that is associated with the stimulation of Th1-biased CD4⁺ T lymphocyte responses (Hope et al., 2000, 2004) and the secretion of IFN- γ by NK cells and $\gamma\delta$ T lymphocytes (J.C. Hope, unpublished). In humans, dendritic cells also promote IFN- γ release from natural killer (NK) T cells, a major source of early IFN- γ in TB pleurisy (Schierloh et al., 2007). In mice infected with BCG, IL-17 is detected after 1 day in the lung (Umemura et al., 2007). Gamma-delta T cells are a primary source of IL-17, following activation with IL-23 (produced by macrophages and dendritic cells). Secretion of IL-17 by $\gamma\delta$ T cells up-regulates Th1 responses, neutrophil inflammatory responses and IFN- γ release by T cells (Umemura et al., 2007).

Lymphocytes in the lungs of patients with pulmonary tuberculosis typically have a Th1 phenotype, secreting IFN- γ (Mazzarella et al., 2003; Taha et al., 1997). However, Th2 lymphocyte subsets have been observed in lung tissue from patients with cavitary tuberculosis, compared with Th1 subsets in non-cavitary disease (Mazzarella et al., 2003). This correlates with higher production of IL-4 in the periphery of patients with cavitary disease (van Crevel et al., 2000) and suggests that IL-4 might be an indicator of disease severity. However, another study has shown that lymphocytes taken from the cavitary wall produce more IFN- γ in response to mycobacterial antigens than cells from non-progressive tuberculoma tissues (Ulrichs et al., 2005). In some cases, granulomas can be positive for both IFN- γ and IL-4 (Fenhalls et al., 2002, 2000). Some authors have reported a Th1 response in the periphery of patients with mild pulmonary tuberculosis and Th2 responses in anergic patients and those with more severe disease (Boussiotis et al., 2000; Dlugovitzky et al., 1997). Clinical observations in tuberculin-reactive patients have also demonstrated that Th1 responses, while high in the granuloma, are often paradoxically depressed in peripheral blood lymphocytes in response to mycobacterial antigens (Jo et al., 2003; Zhang et al., 1995). The phenotype of infected macrophages and dendritic cells within the granuloma is less well defined, but *in vitro* studies in human peripheral blood monocytes/macrophages have demonstrated that virulent strains of tuberculosis preferentially up-regulate the production of Th2 cytokines (IL-4, IL-5, IL-10 and IL-13), while non-virulent strains induce a Th1-type response (Freeman et al., 2006; Manca et al., 2004; Sun et al., 2006). Thus, it is possible that in the typically Th1 environment of the granuloma, localised secretion of IL-4 and IL-13 could specifically impair the response of infected macrophages to IFN- γ . However, secretion of anti-inflammatory cytokines like IL-4, TGF- β and IL-10 may be necessary to dampen the pathological effects of a prolonged or excessive Th1 response, although in progressive tuberculosis disease IL-4 can actually increase immunopathology (Hernandez-Pando et al., 2004; Rook et al., 2004).

Analysis of granuloma development in human lungs have revealed a distinct spatio-temporal sequence of cellular infiltration to sites of mycobacterial infection. The necrotic centre is

surrounded by a layer rich in CD68⁺ antigen-presenting cells (APC), large epithelioid cells and langerhans cells and CD4⁺ and CD45RO⁺ memory cells, but very few CD8⁺ cells (Ulrichs et al., 2004). This is surrounded by an outer layer of follicle-like structures, with mycobacteria-containing CD68⁺ cells and large numbers of CD4⁺ and CD8⁺ cells and B cells (Ulrichs et al., 2004). These follicular structures, resembling secondary lymphoid organs, may be responsible for orchestrating the local immune response to mycobacterial infection (Ulrichs et al., 2004). Thus, within these areas, infected macrophages (and other APC) could be directly and powerfully activated by T cells, particularly through the release of IFN- γ and TNF- α .

3. IFN- γ promotes phagosome maturation in macrophages

Numerous studies have shown that priming macrophages and dendritic cells with IFN- γ prior to infection increases intracellular killing of mycobacteria (see Hope et al., 2004; Russell, 2001). This killing appears to be directed by both nitric oxide synthase 2 (NOS2)-dependent and -independent mechanisms. In mice, IFN- γ -induced expression of NOS2 restricts *M. tuberculosis* replication via the generation of NO. However, NOS2^{-/-} mice infected with *M. tuberculosis* survive significantly longer than mice lacking IFN- γ , IFN- γ R1 or STAT1, through which IFN- γ signals, indicating the existence of IFN- γ -dependent, NOS2-independent immunity against *M. tuberculosis* (MacMicking et al., 2003). One effector in this response is the GTPase Irgm1 (previously called LRG-47), which has specific anti-mycobacterial actions (MacMicking et al., 2003). Although the precise role of Irgm1 in the response to mycobacterial infection is not yet clear, it is involved in phagosome biogenesis (MacMicking et al., 2003).

The success of mycobacteria as intracellular pathogens is due in part to their ability to inhibit maturation of the macrophage phagosome, preventing fusion with lysosomes, while still allowing fusion with transferrin-containing endosomes, thus maintaining a habitable environment that promotes survival (Deretic et al., 2006; Russell, 2001). However, when macrophages are activated with IFN- γ , either alone or in combination with LPS, they are largely able to overcome this phagosome maturation block (Schaible et al., 1998; Via et al., 1998). Following activation with IFN- γ , Irgm1 is recruited to the mycobacterial phagosome, but in Irgm1^{-/-} mice phagosome maturation is impaired (MacMicking et al., 2003). The role of Irgm1 on the phagosome is not known, but it may facilitate fusion with late endosomes or recruitment of IFN- γ -inducible NOS2, a process that is normally inhibited by mycobacteria (MacMicking, 2005; Miller et al., 2004). Recent evidence has also linked Irgm1 with another IFN- γ -induced anti-mycobacterial process; autophagy.

4. Autophagy and the innate immune response to mycobacteria

Macroautophagy (hereafter referred to as autophagy) is a fundamental homeostatic mechanism in which cells sequester discrete portions of the cytoplasm into an autophagosome, a specialized vacuole with a double membrane, which can fuse with lysosomes for degradation (Levine, 2005; Shintani and Klionsky, 2004). This process removes damaged or surplus organelles, such as leaky mitochondria and excess peroxisomes and promotes cell survival by degrading long-lived cytoplasmic macromolecules during periods of starvation (Kuma et al., 2004). Autophagy is controlled by the target of rapamycin (TOR), a conserved Ser/Thr kinase that regulates cell proliferation and metabolism in response to growth factors, energy inputs and nutritional demands (Wullschleger et al., 2006). Activation of TOR stimulates anabolic processes and biomass production, while its inhibition enhances catabolic processes, including autophagy (Fig. 1). For example, treatment with rapamycin or amino acid starvation leads to the inhibition of mTOR and induction of autophagy. Conversely, TOR can be activated by growth factors via the Akt pathway, resulting in the inhibition of autophagy (Wullschleger et al., 2006).

Autophagy has been implicated in the adaptive immune response by playing a role in endogenous antigen presentation (Dengjel et al., 2005; Paludan et al., 2005). Autophagy is also involved in the innate response (Levine and Deretic, 2007). Ligation of TLR4 with LPS induces autophagy in macrophages via a Toll-interleukin-1 receptor domain-containing adaptor-inducing interferon- β (TRIF)-dependent, MyD88-independent mechanism (Xu et al., 2007). In addition, autophagy plays a role in the response to a number of intracellular pathogens, including Epstein-Barr Virus (Paludan et al., 2005), *Shigella flexnari* (Ogawa et al., 2005), *Salmonella typhimurium* (Birmingham et al., 2006) and *Toxoplasma gondii* (Ling et al., 2006). Induction of autophagy by amino acid starvation or treatment with rapamycin in macrophages infected with *M. tuberculosis* or *M. bovis* bacillus Calmette-Guerin (BCG) leads to an increase in phagosome maturation and a decrease in intracellular survival of the bacteria (Gutierrez et al., 2004). Thus, autophagy represents a mechanism by which macrophages are able to overcome the phagosome maturation block imposed by mycobacteria, leading ultimately to enhanced mycobacterial destruction. Moreover, IFN- γ , a key anti-mycobacterial cytokine, has been demonstrated to induce autophagy in infected macrophages (Gutierrez et al., 2004).

5. Autophagy and Th1 – Th2 polarisation

The mechanism by which IFN- γ induces autophagy in macrophages is not yet fully understood. However, it is clear that Irgm1 is involved in this process. Transfection of murine macrophages with Irgm1 promotes the formation of autophagosomes, while siRNA knockdown of Irgm1 inhibits IFN- γ -induced autophagosome formation (Gutierrez et al., 2004; Singh et al., 2006). The human ortholog of Irgm1, IRGM also participates in autophagy. Although not itself IFN- γ -inducible, siRNA knockdown of IRGM in human macrophages inhibits autophagosome formation and maturation of BCG-containing phagosomes, thereby promoting mycobacterial survival (Singh et al., 2006). The extent to which autophagy contributes to IFN- γ -induced phagosome maturation in macrophages infected with mycobacteria has yet to be fully investigated. While mycobacteria-containing autophagosomes can be observed following IFN- γ treatment (Gutierrez et al., 2004; Singh et al., 2006) it is not yet clear whether all IFN- γ -induced phagolysosomes are actually autolysosomes. However, siRNA knockdown of Beclin 1, a key regulator of autophagosome formation, completely abrogates the effect of IFN- γ on phagosome-lysosome fusion in infected murine RAW264.7 macrophages (Harris et al., 2007), suggesting that autophagy may be responsible for the IFN- γ -induced phagosome maturation in these cells.

TNF- α is an important factor in the protective immune response to *M. tuberculosis*; it is essential for the formation and maintenance of the granuloma (Flynn and Chan, 2001). Moreover, TNF- α induces apoptosis in infected cells, depriving the mycobacteria of their niche cell (Fratazzi et al., 1999) and packaging mycobacterial antigens in apoptotic vesicles for uptake and presentation by dendritic cells (Schaible et al., 2003; Winau et al., 2006). A number of studies suggest that TNF- α may also be important in the modulation of autophagy. Autophagic elimination of *Toxoplasma gondii* is induced in infected macrophages following ligation of CD40, coupled with exogenous or autocrine TNF- α signalling (Andrade et al., 2006; Ling et al., 2006). In T lymphoblastic leukemic cell lines autophagy has been observed at an early stage of TNF- α -induced cell death (Jia et al., 1997). Treatment of human atherosclerotic vascular smooth cells with TNF- α has been shown to induce expression of the autophagy genes MAPLC3 and Beclin 1 and this effect is dependent on activation of the Jun kinase (JNK) pathway (Jia et al., 2006). In addition, TNF- α might also promote autophagy by inhibiting activation of the Akt pathway (Jia et al., 2006). Activation of the Akt pathway inhibits autophagy via activation of mTOR, while siRNA-mediated knockdown of Akt induces autophagy in murine macrophages (Harris et al., 2007). In MCF-7 human breast cancer cells, TNF- α -induced autophagy is dependent on ERK1/2 signalling and inhibition of ERK signalling

results in greater sensitivity to TNF- α -induced cell death (Sivaprasad and Basu, 2008). In Ewing sarcoma cells, TNF- α -induced autophagy is inhibited by activation of NF- κ B and is dependent on the production of reactive oxygen species (Djavaheri-Mergny et al., 2006). *Mycobacterium tuberculosis* can inhibit TNF- α -induced apoptosis by activating NF- κ B and enhancing the production of soluble TNF receptor 2 (Gao and Kwaik, 2000). These may also represent strategies to inhibit TNF- α -induced autophagy.

While IFN- γ and TNF- α stimulate autophagy, the archetypal Th2 cytokines IL-4 and IL-13 have the opposite effect. In HT-29 epithelial cells, for example, IL-13 is a potent inhibitor of starvation-induced autophagy via stimulation of the Akt pathway (Arico et al., 2001; Petiot et al., 2000). Both IL-4 and IL-13, signal through IL-4R α , which forms a heterodimer with gamma common (γ c) chain (for the IL-4 receptor) or the IL-13R α 1 (for the IL-13 receptor) (Nelms et al., 1999). Ligation of these receptor complexes results in signalling via the insulin receptor substrate (IRS)-1/2 and STAT6 pathways (Nelms et al., 1999). While STAT6 is involved in IL-4 and IL-13-induced gene expression, IRS-1/2 signalling activates the type I phosphatidylinositol 3-kinase (PI3K) pathway and subsequently the Akt pathway (Fig. 1). In human and murine macrophages infected with mycobacteria, both IL-4 and IL-13 inhibit starvation-induced autophagy through activation of the Akt pathway (Harris et al., 2007). This results in decreased phagosome maturation and promotes intracellular survival of the bacteria. Similarly, IL-4 and IL-13 both inhibit IFN- γ -induced autophagy, but this process is independent of Akt; instead it is dependent on STAT6 signalling (Harris et al., 2007).

These data suggest that autophagy, as well as being a potentially important anti-mycobacterial response in macrophages, may be mediated by Th1 and Th2 cytokines. The predominantly Th1 environment within the lungs of patients/cattle with active tuberculosis should promote intracellular killing of mycobacteria by NOS2-dependent and -independent mechanisms, including autophagy. Moreover, autophagy may represent a means of targeting bacteria to phagolysosomes (or autolysosomes) for processing and presentation of antigens and thus could play an important role in control of mycobacteria within the granuloma and help to drive the adaptive immune response (Fig. 2). Conversely, IL-4 and IL-13, released by macrophages infected with virulent strains of *M. tuberculosis* (Freeman et al., 2006; Manca et al., 2004; Sun et al., 2006), could act in an autocrine manner to inhibit the autophagic process.

6. Conclusions and future directions

It has not yet been determined whether autophagy contributes significantly to the immune response against mycobacteria *in vivo*, although the potential importance of this process is clear. In this context, it would be interesting to study the autophagic status of alveolar macrophages and dendritic cells during pulmonary tuberculosis, both within and outside the granuloma. The role of various different cytokines on this process may prove insightful, particularly those of potential importance in the innate immune response and granuloma formation, such as IL-10 and TGF- β . It is notable that at least two intracellular pathogens, *Listeria monocytogenes* and *Legionella pneumophila*, have evolved mechanisms to evade or subvert the autophagic pathway and increase their own survival (Birmingham et al., 2007; Dubuisson and Swanson, 2006). Whether mycobacteria are capable of modulating autophagosome formation and maturation, as they are with phagosome maturation, could be particularly interesting and provide further insight into the evolutionary arms race between pathogen and host.

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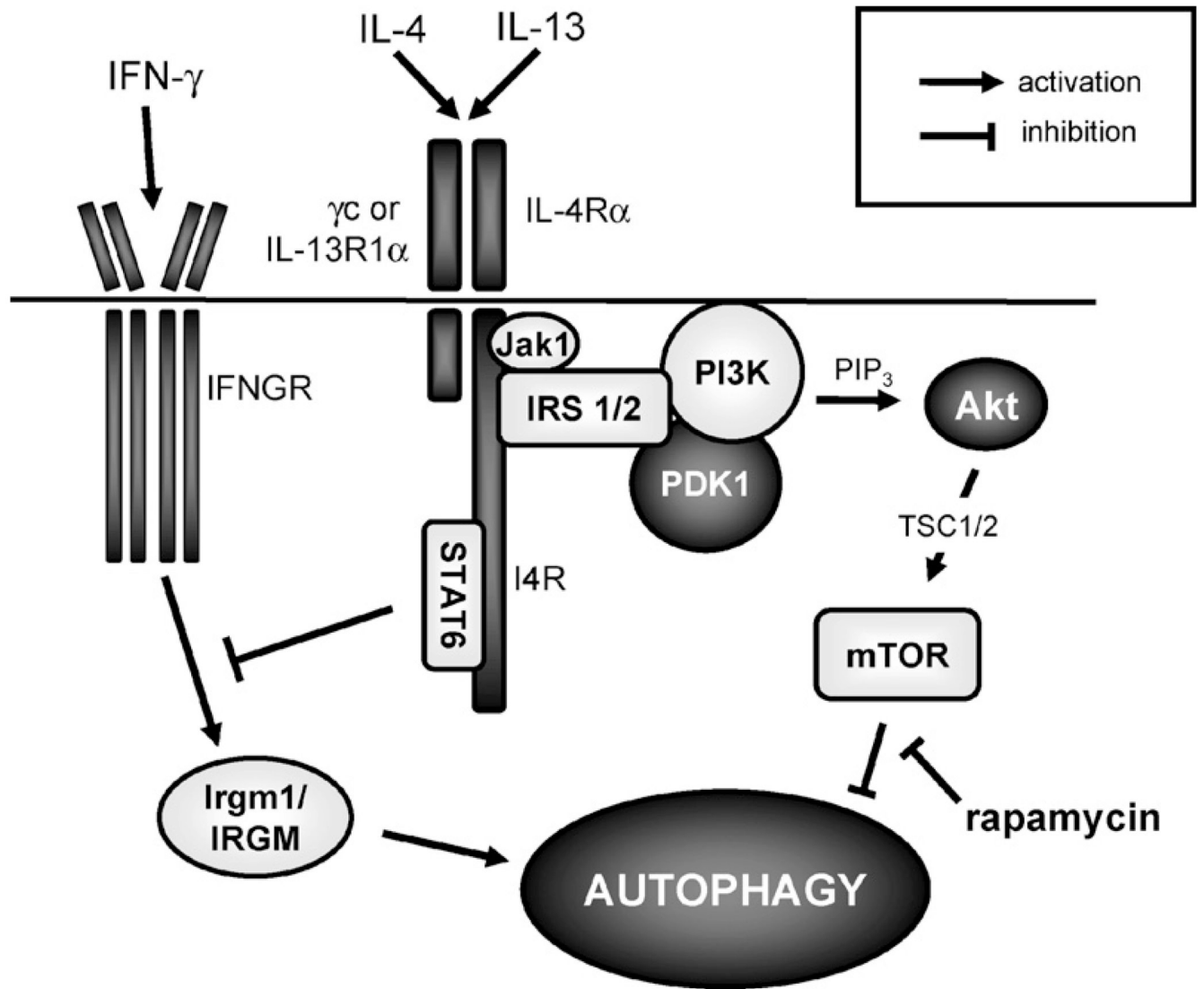
References

- Andrade RM, Wessendarp M, Gubbels MJ, Striepen B, Subauste CS. CD40 induces macrophage anti-Toxoplasma gondii activity by triggering autophagy-dependent fusion of pathogen-containing vacuoles and lysosomes. *J. Clin. Invest* 2006;116:2366–2377. [PubMed: 16955139]
- Arico S, Petiot A, Bauvy C, Dubbelhuis PF, Meijer AJ, Codogno P, Ogier-Denis E. The tumor suppressor PTEN positively regulates macroautophagy by inhibiting the phosphatidylinositol 3-kinase/protein kinase B pathway. *J. Biol. Chem* 2001;276:35243–35246. [PubMed: 11477064]
- Birmingham CL, Canadien V, Gouin E, Troy EB, Yoshimori T, Cossart P, Higgins DE, Brumell JH. *Listeria monocytogenes* evades killing by autophagy during colonization of host cells. *Autophagy* 3. 2007
- Birmingham CL, Smith AC, Bakowski MA, Yoshimori T, Brumell JH. Autophagy controls Salmonella infection in response to damage to the Salmonella-containing vacuole. *J. Biol. Chem* 2006;281:11374–11383. [PubMed: 16495224]
- Boussiotis VA, Tsai EY, Yunis EJ, Thim S, Delgado JC, Dascher CC, Berezovskaya A, Rousset D, Reynes JM, Goldfeld AE. IL-10-producing T cells suppress immune responses in anergic tuberculosis patients. *J. Clin. Invest* 2000;105:1317–1325. [PubMed: 10792007]
- de Jong R, Altare F, Haagen IA, Elferink DG, Boer T, van Breda Vriesman PJ, Kabel PJ, Draaisma JM, van Dissel JT, Kroon FP, Casanova JL, Ottenhoff TH. Severe mycobacterial and Salmonella infections in interleukin-12 receptor-deficient patients. *Science* 1998;280:1435–1438. [PubMed: 9603733]
- Dengjel J, Schoor O, Fischer R, Reich M, Kraus M, Muller M, Kreymborg K, Altenberend F, Brandenburg J, Kalbacher H, Brock R, Driessen C, Rammensee HG, Stevanovic S. Autophagy promotes MHC class II presentation of peptides from intracellular source proteins. *Proc. Natl. Acad. Sci. U.S.A* 2005;102:7922–7927. [PubMed: 15894616]
- Deretic V, Singh S, Master S, Harris J, Roberts E, Kyei G, Davis A, de Haro S, Naylor J, Lee HH, Vergne I. *Mycobacterium tuberculosis* inhibition of phagolysosome biogenesis and autophagy as a host defence mechanism. *Cell Microbiol* 2006;8:719–727. [PubMed: 16611222]
- Djavaheri-Mergny M, Amelotti M, Mathieu J, Besancon F, Bauvy C, Souquere S, Pierron G, Codogno P. NF-kappaB activation represses tumor necrosis factor-alpha-induced autophagy. *J. Biol. Chem* 2006;281:30373–30382. [PubMed: 16857678]
- Dlugovitzky D, Torres-Morales A, Ratani L, Farroni MA, Largacha C, Molteni O, Bottasso O. Circulating profile of Th1 and Th2 cytokines in tuberculosis patients with different degrees of pulmonary involvement. *FEMS Immunol. Med. Microbiol* 1997;18:203–207. [PubMed: 9271171]
- Dubuisson JF, Swanson MS. Mouse infection by Legionella, a model to analyze autophagy. *Autophagy* 2 2006:179–182.
- Fenhalls G, Stevens L, Bezuidenhout J, Amphlett GE, Duncan K, Bardin P, Lukey PT. Distribution of IFN-gamma, IL-4 and TNF-alpha protein and CD8 T cells producing IL-12p40 mRNA in human lung tuberculous granulomas. *Immunology* 2002;105:325–335. [PubMed: 11918694]
- Fenhalls G, Wong A, Bezuidenhout J, van Helden P, Bardin P, Lukey PT. In situ production of gamma interferon, interleukin-4, and tumor necrosis factor alpha mRNA in human lung tuberculous granulomas. *Infect. Immun* 2000;68:2827–2836. [PubMed: 10768979]
- Ferlazzo G, Morandi B, D'Agostino A, Meazza R, Melioli G, Moretta A, Moretta L. The interaction between NK cells and dendritic cells in bacterial infections results in rapid induction of NK cell activation and in the lysis of uninfected dendritic cells. *Eur. J. Immunol* 2003;33:306–313. [PubMed: 12548561]
- Flynn JL, Chan J. Immunology of tuberculosis. *Annu. Rev. Immunol* 2001;19:93–129. [PubMed: 11244032]
- Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. An essential role for interferon gamma in resistance to Mycobacterium tuberculosis infection. *J. Exp. Med* 1993;178:2249–2254. [PubMed: 7504064]
- Flynn JL, Goldstein MM, Triebold KJ, Sypek J, Wolf S, Bloom BR. IL-12 increases resistance of BALB/c mice to Mycobacterium tuberculosis infection. *J. Immunol* 1995;155:2515–2524. [PubMed: 7650381]

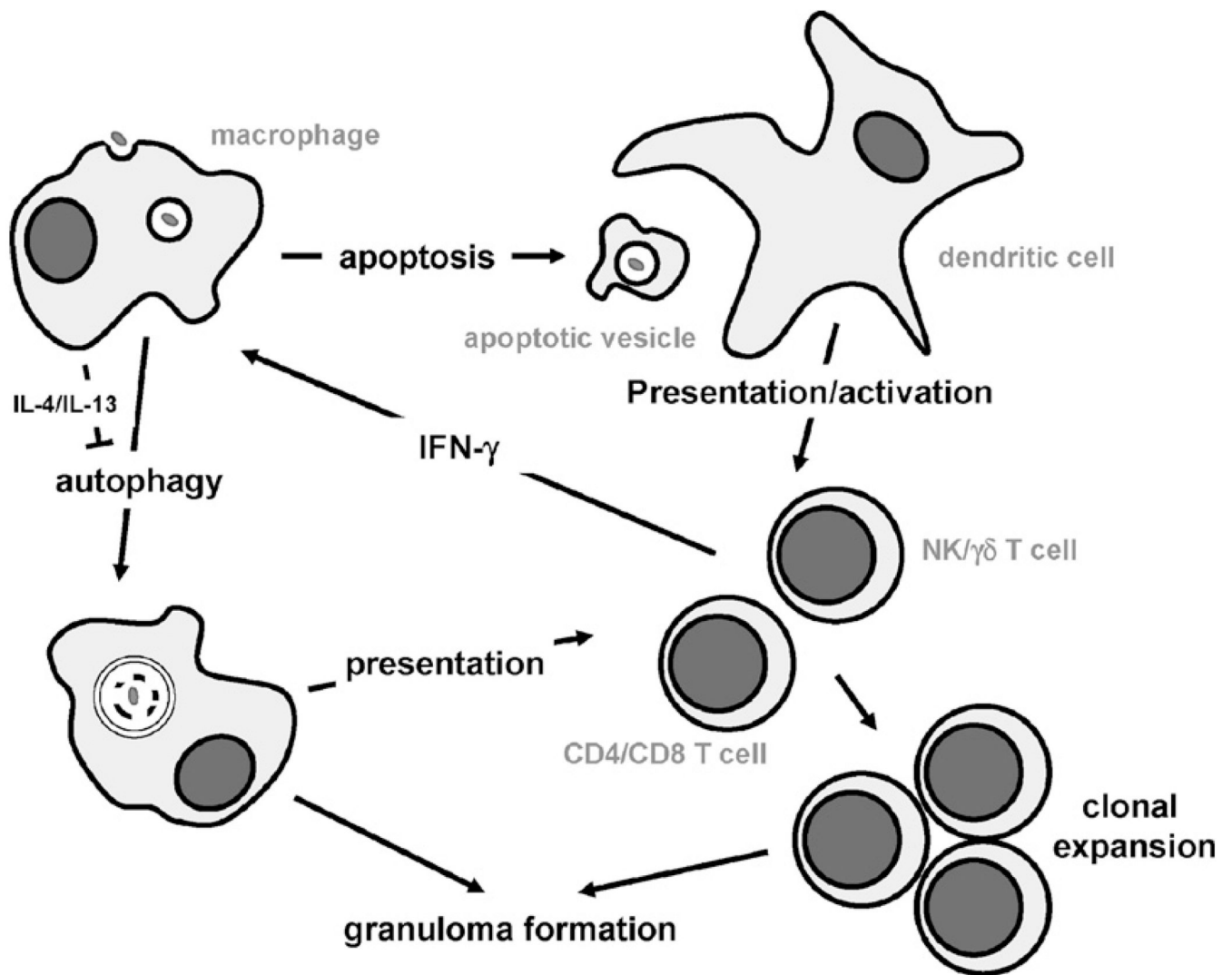
- Fratazzi C, Arbeit RD, Carini C, Balcewicz-Sablinska MK, Keane J, Kornfeld H, Remold HG. Macrophage apoptosis in mycobacterial infections. *J. Leukoc. Biol* 1999;66:763–764. [PubMed: 10577507]
- Freeman S, Post FA, Bekker LG, Harbacheuski R, Steyn LM, Ryffel B, Connell ND, Kreiswirth BN, Kaplan G. *Mycobacterium tuberculosis* H37Ra and H37Rv differential growth and cytokine/chemokine induction in murine macrophages in vitro. *J. Interferon Cytokine Res* 2006;26:27–33. [PubMed: 16426145]
- Gao LY, Kwaik YA. The modulation of host cell apoptosis by intracellular bacterial pathogens. *Trends Microbiol* 2000;8:306–313. [PubMed: 10878765]
- Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. *Cell* 2004;119:753–766. [PubMed: 15607973]
- Harris J, De Haro SA, Master SS, Keane J, Roberts EA, Delgado M, Deretic V. T helper 2 cytokines inhibit autophagic control of intracellular *Mycobacterium tuberculosis*. *Immunity* 2007;27:505–517. [PubMed: 17892853]
- Hernandez-Pando R, Aguilar D, Hernandez ML, Orozco H, Rook G. Pulmonary tuberculosis in BALB/c mice with non-functional IL-4 genes: changes in the inflammatory effects of TNF-alpha and in the regulation of fibrosis. *Eur. J. Immunol* 2004;34:174–183. [PubMed: 14971043]
- Hope JC, Kwong LS, Sopp P, Collins RA, Howard CJ. Dendritic cells induce CD4+ and CD8+ T-cell responses to *Mycobacterium bovis* and *M. avium* antigens in Bacille Calmette Guerin vaccinated and nonvaccinated cattle. *Scand. J. Immunol* 2000;52:285–291. [PubMed: 10972905]
- Hope JC, Thom ML, McCormick PA, Howard CJ. Interaction of antigen presenting cells with mycobacteria. *Vet. Immunol. Immunopathol* 2004;100:187–195. [PubMed: 15207456]
- Hope JC, Vordermeier HM. Vaccines for bovine tuberculosis: current views and future prospects. *Expert Rev. Vaccines* 2005;4:891–903. [PubMed: 16372884]
- Jia G, Cheng G, Gangahar DM, Agrawal DK. Insulin-like growth factor-1 and TNF-alpha regulate autophagy through c-jun N-terminal kinase and Akt pathways in human atherosclerotic vascular smooth cells. *Immunol. Cell Biol* 2006;84:448–454.
- Jia L, Dourmashkin RR, Allen PD, Gray AB, Newland AC, Kelsey SM. Inhibition of autophagy abrogates tumour necrosis factor alpha induced apoptosis in human T-lymphoblastic leukaemic cells. *Br. J. Haematol* 1997;98:673–685. [PubMed: 9332326]
- Jo EK, Park JK, Dockrell HM. Dynamics of cytokine generation in patients with active pulmonary tuberculosis. *Curr. Opin. Infect. Dis* 2003;16:205–210. [PubMed: 12821809]
- Jouanguy E, Altare F, Lamhamedi S, Revy P, Emile JF, Newport M, Levin M, Blanche S, Seboun E, Fischer A, Casanova JL. Interferon-gamma-receptor deficiency in an infant with fatal bacille Calmette-Guerin infection. *N. Engl. J. Med* 1996;335:1956–1961. [PubMed: 8960475]
- Jouanguy E, Lamhamedi-Cherradi S, Lammas D, Dorman SE, Fondaneche MC, Dupuis S, Doffinger R, Altare F, Girdlestone J, Emile JF, Ducoulombier H, Edgar D, Clarke J, Oxelius VA, Brai M, Novelli V, Heyne K, Fischer A, Holland SM, Kumararatne DS, Schreiber RD, Casanova JL. A human IFNGR1 small deletion hotspot associated with dominant susceptibility to mycobacterial infection. *Nat. Genet* 1999;21:370–378. [PubMed: 10192386]
- Keane J. TNF-blocking agents and tuberculosis: new drugs illuminate an old topic. *Rheumatology (Oxford)* 2005;44:714–720. [PubMed: 15741198]
- Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwiertman WD, Siegel JN, Braun MM. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N. Engl. J. Med* 2001;345:1098–1104. [PubMed: 11596589]
- Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, Ohsumi Y, Tokuhiya T, Mizushima N. The role of autophagy during the early neonatal starvation period. *Nature* 2004;432:1032–1036. [PubMed: 15525940]
- Levine B. Eating oneself and uninvited guests: autophagy-related pathways in cellular defense. *Cell* 2005;120:159–162. [PubMed: 15680321]
- Levine B, Deretic V. Unveiling the roles of autophagy in innate and adaptive immunity. *Nat. Rev. Immunol* 2007;7:767–777. [PubMed: 17767194]

- Ling YM, Shaw MH, Ayala C, Coppens I, Taylor GA, Ferguson DJ, Yap GS. Vacuolar and plasma membrane stripping and autophagic elimination of *Toxoplasma gondii* in primed effector macrophages. *J. Exp. Med* 2006;203:2063–2071. [PubMed: 16940170]
- MacMicking JD. Immune control of phagosomal bacteria by p47 GTPases. *Curr. Opin. Microbiol* 2005;8:74–82. [PubMed: 15694860]
- MacMicking JD, Taylor GA, McKinney JD. Immune control of tuberculosis by IFN-gamma-inducible LRG-47. *Science* 2003;302:654–659. [PubMed: 14576437]
- Manca C, Reed MB, Freeman S, Mathema B, Kreiswirth B, Barry CE 3rd, Kaplan G. Differential monocyte activation underlies strain-specific *Mycobacterium tuberculosis* pathogenesis. *Infect. Immun* 2004;72:5511–5514. [PubMed: 15322056]
- Mazzarella G, Bianco A, Perna F, D'Auria D, Grella E, Moscariello E, Sanduzzi A. T lymphocyte phenotypic profile in lung segments affected by cavitary and non-cavitary tuberculosis. *Clin. Exp. Immunol* 2003;132:283–288. [PubMed: 12699418]
- Miller BH, Fratti RA, Poschet JF, Timmins GS, Master SS, Burgos M, Marletta MA, Deretic V. *Mycobacteria* inhibit nitric oxide synthase recruitment to phagosomes during macrophage infection. *Infect. Immun* 2004;72:2872–2878. [PubMed: 15102799]
- Nelms K, Keegan AD, Zamorano J, Ryan JJ, Paul WE. The IL-4 receptor: signaling mechanisms and biologic functions. *Annu. Rev. Immunol* 1999;17:701–738. [PubMed: 10358772]
- Nobukuni T, Kozma SC, Thomas G. hvps34, an ancient player, enters a growing game: mTOR Complex 1/S6K1 signaling. *Curr. Opin. Cell Biol* 2007;19:135–141. [PubMed: 17321123]
- Ogawa M, Yoshimori T, Suzuki T, Sagara H, Mizushima N, Sasakawa C. Escape of intracellular *Shigella* from autophagy. *Science* 2005;307:727–731. [PubMed: 15576571]
- Paludan C, Schmid D, Landthaler M, Vockerodt M, Kube D, Tuschl T, Munz C. Endogenous MHC class II processing of a viral nuclear antigen after autophagy. *Science* 2005;307:593–596. [PubMed: 15591165]
- Petiot A, Ogier-Denis E, Blommaert EF, Meijer AJ, Codogno P. Distinct classes of phosphatidylinositol 3'-kinases are involved in signaling pathways that control macroautophagy in HT-29 cells. *J. Biol. Chem* 2000;275:992–998. [PubMed: 10625637]
- Rook GA, Hernandez-Pando R, Dheda K, Teng Seah G. IL-4 in tuberculosis: implications for vaccine design. *Trends Immunol* 2004;25:483–488. [PubMed: 15324741]
- Russell DG. *Mycobacterium tuberculosis*: here today, and here tomorrow. *Nat. Rev. Mol. Cell Biol* 2001;2:569–577. [PubMed: 11483990]
- Salgame P. Host innate and Th1 responses and the bacterial factors that control *Mycobacterium tuberculosis* infection. *Curr. Opin. Immunol* 2005;17:374–380. [PubMed: 15963709]
- Schaible UE, Sturgill-Koszycki S, Schlesinger PH, Russell DG. Cytokine activation leads to acidification and increases maturation of *Mycobacterium avium*-containing phagosomes in murine macrophages. *J. Immunol* 1998;160:1290–1296. [PubMed: 9570546]
- Schaible UE, Winau F, Sieling PA, Fischer K, Collins HL, Hagens K, Modlin RL, Brinkmann V, Kaufmann SH. Apoptosis facilitates antigen presentation to T lymphocytes through MHC-I and CD1 in tuberculosis. *Nat. Med* 2003;9:1039–1046. [PubMed: 12872166]
- Schierloh P, Yokobori N, Aleman M, Landoni V, Geffner L, Musella RM, Castagnino J, Baldini M, Abbate E, de la Barrera SS, Sasiain MC. *Mycobacterium tuberculosis*-induced gamma interferon production by natural killer cells requires cross talk with antigen-presenting cells involving toll-like receptors 2 and 4 and the mannose receptor in tuberculous pleurisy. *Infect. Immun* 2007;75:5325–5337. [PubMed: 17709420]
- Schmid D, Pypaert M, Munz C. Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. *Immunity* 2007;26:79–92. [PubMed: 17182262]
- Shintani T, Klionsky DJ. Autophagy in health and disease: a double-edged sword. *Science* 2004;306:990–995. [PubMed: 15528435]
- Shrestha N, Ida JA, Lubinski AS, Pallin M, Kaplan G, Haslett PA. Regulation of acquired immunity by gamma delta T-cell/dendritic-cell interactions. *Ann. N. Y. Acad. Sci* 2005;1062:79–94. [PubMed: 16461791]

- Singh SB, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science* 2006;313:1438–1441. [PubMed: 16888103]
- Sivaprasad U, Basu A. Inhibition Of ERK attenuates autophagy and potentiates tumor necrosis factor- α -induced cell death in MCF-7 cells. *J. Cell Mol. Med.* 2008
- Sun YJ, Lim TK, Ong AK, Ho BC, Seah GT, Paton NI. Tuberculosis associated with *Mycobacterium tuberculosis* Beijing and non-Beijing genotypes: a clinical and immunological comparison. *BMC Infect. Dis* 2006;6:105. [PubMed: 16820066]
- Taha RA, Kotsimbos TC, Song YL, Menzies D, Hamid Q. IFN- γ and IL-12 are increased in active compared with inactive tuberculosis. *Am. J. Respir. Crit. Care Med* 1997;155:1135–1139. [PubMed: 9116999]
- Ulrichs T, Kosmiadi GA, Jorg S, Pradl L, Titukhina M, Mishenko V, Gushina N, Kaufmann SH. Differential organization of the local immune response in patients with active cavitary tuberculosis or with nonprogressive tuberculoma. *J. Infect. Dis* 2005;192:89–97. [PubMed: 15942898]
- Ulrichs T, Kosmiadi GA, Trusov V, Jorg S, Pradl L, Titukhina M, Mishenko V, Gushina N, Kaufmann SH. Human tuberculous granulomas induce peripheral lymphoid follicle-like structures to orchestrate local host defence in the lung. *J. Pathol* 2004;204:217–228. [PubMed: 15376257]
- Umemura M, Yahagi A, Hamada S, Begum MD, Watanabe H, Kawakami K, Suda T, Sudo K, Nakae S, Iwakura Y, Matsuzaki G. IL-17-mediated regulation of innate and acquired immune response against pulmonary *Mycobacterium bovis* bacille Calmette-Guerin infection. *J. Immunol* 2007;178:3786–3796. [PubMed: 17339477]
- van Crevel R, Karyadi E, Preyers F, Leenders M, Kullberg BJ, Nelwan RH, van der Meer JW. Increased production of interleukin 4 by CD4+ and CD8+ T cells from patients with tuberculosis is related to the presence of pulmonary cavities. *J. Infect. Dis* 2000;181:1194–1197. [PubMed: 10720554]
- Via LE, Fratti RA, McFalone M, Pagan-Ramos E, Deretic D, Deretic V. Effects of cytokines on mycobacterial phagosome maturation. *J. Cell Sci* 1998;111(Pt 7):897–905. [PubMed: 9490634]
- Winau F, Weber S, Sad S, de Diego J, Hoops SL, Breiden B, Sandhoff K, Brinkmann V, Kaufmann SH, Schaible UE. Apoptotic vesicles crossprime CD8 T cells and protect against tuberculosis. *Immunity* 2006;24:105–117. [PubMed: 16413927]
- Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell* 2006;124:471–484. [PubMed: 16469695]
- Xu Y, Jagannath C, Liu XD, Sharafkhaneh A, Kolodziejaska KE, Eissa NT. Toll-like receptor 4 is a sensor for autophagy associated with innate immunity. *Immunity* 2007;27:135–144. [PubMed: 17658277]
- Zhang M, Lin Y, Iyer DV, Gong J, Abrams JS, Barnes PF. T-cell cytokine responses in human infection with *Mycobacterium tuberculosis*. *Infect. Immun* 1995;63:3231–3234. [PubMed: 7622255]

**Fig. 1.**

Cytokine signaling and autophagy. Following ligation of the IL-4/IL-13 receptor, the insulin receptor substrate (IRS 1/2) interacts with the phosphorylated I4R motif of the IL-4R α via Jak1. Type III PI3K and PDK1 are recruited to the complex, leading to the formation of phosphoinositides (including PIP₃), which in turn phosphorylate Akt. Through its interaction with the tuberous sclerosis protein 1 and 2 complex (TSC1/2), Akt activates mammalian target of rapamycin (mTOR), which inhibits autophagy (Nobukuni et al., 2007). Inhibition of mTOR, such as by rapamycin, induces autophagy. IFN- γ -induced autophagy, dependent on Irgm1 (IRGM in humans), is inhibited by Th2 cytokines via STAT6 activation.

**Fig. 2.**

Autophagy has the potential to play an important role in the immune response to mycobacteria. In this hypothesised model of granuloma formation, alveolar macrophages, unable to overcome the phagosome maturation block imposed by infecting mycobacteria, undergo apoptosis. Apoptotic vesicles carry mycobacterial protein and glycolipid antigens to dendritic cells (DC) which can prime MHC class I- and CD1-restricted T cells (Schaible et al., 2003; Winau et al., 2006) which, in turn, produce IFN- γ . Moreover, infected DC, through stimulation of NK cells and $\gamma\delta$ T cells, can increase IFN- γ production (Ferlazzo et al., 2003; Shrestha et al., 2005). This activates anti-microbial responses in macrophages, including autophagy, promoting degradation of intracellular mycobacteria and processing and presentation of peptide antigens via MHC Class II to T cells (Schmid et al., 2007). Following clonal expansion, antigen-specific T cells migrate to the site of infection and help to drive the formation of a granuloma. Inhibition of autophagy through the autocrine secretion of IL-4 and/or IL-13 by infected macrophages could potentially disrupt this process, allowing the bacteria to gain a foothold before the formation of a protective granuloma.